

A QUANTITATIVE STUDY OF THE RELATIONSHIP BETWEEN THE ACTIVITY AND OXYGEN CONSUMPTION OF THE GOLDFISH, AND ITS APPLICATION TO THE MEASUREMENT OF RESPIRATORY METABOLISM IN FISHES

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INTRODUCTION

The fact that fish consume more oxygen when active than when quiescent has been observed by many investigators (Krogh, 1916; Bowen, 1932; Clausen, 1933, 1936; Wells, 1935; Schlaifer, 1938; Smith and Matthews, 1942), but apparently no attempt has been made to determine the exact relationship between oxygen consumption and activity in fishes. It is the purpose of this paper to present data which are believed to provide an objective and quantitative basis for the relationship between activity and oxygen consumption in the goldfish, and to describe a method for making the necessary measurements. The method is based on the use of a recording activity detector (Spoor, 1941) combined with a continuous flow system for measuring oxygen consumption.

The lack of definite information on the activity of fish under experimental conditions has been one of the chief sources of difficulty in work on the respiratory metabolism of fishes, and attention has been called to the need for an experimental method which would make it possible to distinguish between "standard metabolism" and the increased metabolism due to muscular movements (Wells, 1935). In view of the fact that the oxygen consumption is affected by changes in the basal metabolic rate as well as by changes in activity, the importance of such a method is apparent. The method employed in the present work seems to meet this need, inasmuch as the state of activity is recorded continuously and periods of inactivity can be selected for measuring basal oxygen consumption.

Szymanski (1914) and Spencer (1939), using other types of activity detectors, have reported that goldfish show considerable individual variation in activity and that the activity pattern is affected by light. Spencer (1939) also found activity to be influenced by food. Knowledge of the behavior of the fish under the experimental conditions is of importance in the collection of data on oxygen consumption in the method to be described, as well as in the interpretation of these data. For this reason further observations on the patterns and rates of activity and on the effects of food, light and disturbances are included in the present paper.

THE ACTIVITY OF THE GOLDFISH UNDER EXPERIMENTAL CONDITIONS

Method

Several dozen goldfish (*Carassius auratus*) ranging between 24 and 96 grams in weight were selected at random from a stock obtained from a local goldfish farm

and studied individually in experimental chambers, each of which was equipped with a recording activity detector. The experimental chambers were set up in a ground floor aquarium room which was seldom entered except for the purposes of this study, so that the fish could be left for long periods with relatively little disturbance. The recording apparatus was kept in another room. Records of the activity of each fish were started shortly after its introduction into a chamber and continued for periods ranging from a few days to many months in length, during which the patterns and rates of activity and the effects of food, light and disturbances upon them were studied. With a few exceptions, oxygen consumption was not measured in this series of observations.

The experimental chamber (Fig. 1) consisted of a one-gallon brown glazed

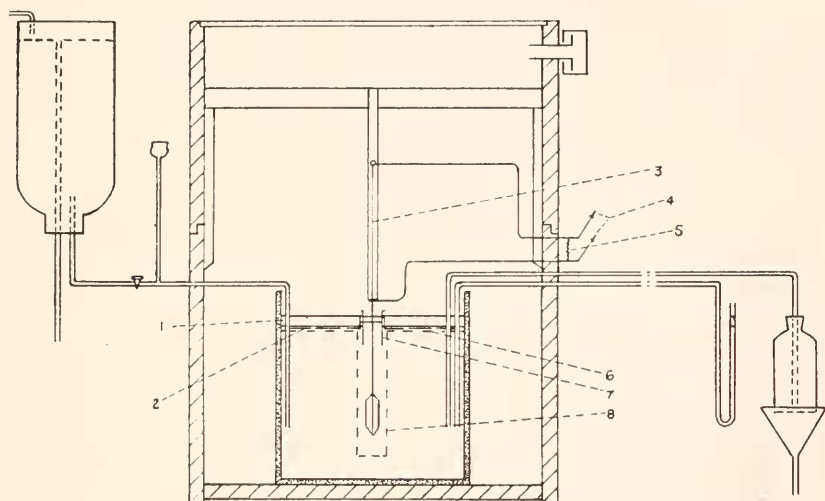


FIGURE 1. Diagram of apparatus for measuring oxygen consumption and activity. (1) paraffin oil (this was omitted when activity alone was being measured), (2) glass plates, (3) No. 44 copper wire, (4) to sensitive relay, (5) resistor, (6) wire screen, (7) glass tube, (8) wire frame protecting paddle. Explanation in text.

crook fitted with a galvanized iron wire screen of $\frac{1}{2}$ inch mesh to prevent the fish from reaching the surface of the water. A glass tube about 3 cm. in diameter was fitted into an opening in the center of the screen so that it extended 3.5 cm. above and 3 cm. below the screen; its purpose will be considered in a later section. The surface of the water stood about 3 mm. above the screen, the total volume to this level being 2,600 cc. Water entered the chamber from a constant level reservoir through 8 mm. glass tubing and left by way of a siphon of 8 mm. glass tubing which dipped into a constant level drain, the rate of flow (between 70 and 100 cc. a minute) being regulated by means of a glass stopcock in the inlet. The intake of the siphon was placed about 5 cm. above the bottom of the chamber, so that feces and other debris that fell to the bottom did not enter the siphon until they had been broken into small pieces in the course of their passage upward to the intake. The chamber was practically self cleaning under these conditions, the flow of the water and the movements of the fish being sufficient to move debris into the siphon. The fish

could therefore be maintained in the chamber for months without cleaning. A thistle tube entering the inlet provided for the introduction of food, being closed off at all other times. The water supply consisted of tap water passed through an activated charcoal filter, brought to the desired temperature and aerated until it approached equilibrium with the atmosphere. Most of the observations were made at temperatures between 20 and 24° C. The fish seldom extracted more than one-third of the oxygen from the water at the rates of flow employed, and they usually took less than this. In view of the findings of Crozier and Stier (1925), Toryu (1927), and Schlaifer (1938), it seems unlikely that behavior was influenced by the oxygen tension of the water.

The chamber was enclosed in a wooden case to minimize disturbances and to make it possible to control the light. The top of the case was fitted with a pane of glass for natural illumination, and with a wooden cover when either complete darkness or constant light was desired. A ventilator in the side of the case, with baffles to prevent light from entering, permitted some circulation of the air. The water inlet, outlet siphon, and a tube leading to a U tube indicating the water level in the chamber passed through the wall; a coat of black paint over each tube prevented light from entering the chamber through these openings.

The detector consisted of a light-weight aluminum paddle suspended in the water in the experimental chamber by a fine copper wire in such a way that a silver rod at the top of the paddle shaft passed through a small hole in a fixed silver plate. Water currents set up by the movements of the fish moved the paddle, causing the rod to make and break contact with the sides of the hole and thus to activate a sensitive relay. This relay operated the recording apparatus. The blade of the paddle consisted of aluminum foil (5 cm. long and 2.5 cm. wide) with the corners bent in at right angles so that the water currents struck a flat surface regardless of their points of origin. The shaft (10 cm. long) consisted of no. 22 aluminum wire cemented to the blade and imbedded at its upper end (7 cm. above the blade) in a bakelite insulating rod (2 cm. long and 0.2 cm. in diameter) in the upper end of which the silver rod (1 cm. long and about 0.04 cm. in diameter) was imbedded. This silver rod was soldered to a 14.5 cm. length of no. 44 enameled copper wire held in an insulated binding post attached to a wooden supporting shaft. A wooden bracket rising from the case supported this shaft in a vertical position so that the paddle hung in the water through the glass tube in the center of the screen. A cylindrical frame of galvanized iron wire protected the paddle from contact with the fish. A small lead weight (about 0.1 gm.) clamped to the paddle shaft below the bakelite helped to bring the paddle back to the resting position after displacement by the water currents. The silver plate (about 0.5 cm. square) was attached to the supporting shaft and held in a horizontal position about 6 cm. above the screen. The hole in the plate was between 0.08 and 0.1 cm. in diameter. The current to operate the sensitive relay was supplied by a 6 volt storage battery; the coil of the relay had a resistance of 1,000 ohms. A 5,000 ohm resistor across the detector contacts prevented sparking and welding without causing an observable reduction in the sensitivity of the detector. The plate was kept warm by means of a small insulated heating coil in order to prevent water from condensing upon it from the humid atmosphere above the chamber.

The sensitivity of the detector could be controlled somewhat by adjusting the position of the silver rod with respect to the sides of the hole in the plate. The nor-

mal movements of the operculum and the position-maintaining fin movements of a quiescent 25- to 30-gram goldfish were usually sufficient to move the paddle slightly, and when the rod was close to the plate these movements were recorded. For the observations to be described, however, the rod was centered in the hole so that the ordinary respiratory movements did not move the paddle enough to make contact, these movements being considered as among the basal functions of the fish. Vigorous respiratory movements and any movement that resulted in a change in the position of the fish moved the paddle enough to make and break contact, slow swimming movements causing few, and vigorous activity causing many impulses to be recorded. At the flow rates used in these experiments the flow of water through the chamber did not move the paddle.

The sensitive relay activated a counter which in turn caused signal magnets to record every tenth and hundredth impulse on a long paper kymograph moving about 30 mm. an hour. The frequency of the impulses was such (ranging up to 6,000 an hour) that they usually could not be counted when recorded individually. The counter was capable of following and recording at least 10 impulses a second. Time was recorded in hours beneath the activity record.

Patterns and rates of activity

In agreement with the results of Szymanski (1914) and Spencer (1939), the goldfish used in this study proved to be quite variable in their patterns and rates of activity, even when they were maintained under almost identical conditions of light, feeding, temperature, water supply and disturbance. Three general types of behavior appeared when the fish were kept under natural conditions of light: (1) arrhythmic activity, in which no relation to day or night could be detected; (2) rhythmic activity, in which the fish were active by day and quiescent at night; (3) rhythmic activity, in which they were quiescent by day and active at night.

Fish showing the first, arrhythmic, type of behavior were extremely variable. A few were vigorously active day and night for periods as long as ten days, others were moderately active throughout the 24-hour period for weeks at a time, and still others remained practically inert for similar periods. Some of these arrhythmic fish, particularly those in the last group, showed irregular bursts of activity now and then, with no apparent relation to the time of day, feeding, or disturbance.

An example of the second type of behavior, diurnal activity and nocturnal quiescence, is shown in Figure 2, which is based on the number of impulses recorded by a 35-gram male goldfish during each hour between 1 P.M. January 11 and 1 P.M. January 13, 1946. The fish was fed at 11:05 A.M. on January 12, otherwise the room was not entered between 2:45 P.M. January 11 and 7:15 P.M. January 13. Aside from the feeding, the effects of which are discussed below, light was the only known variable, the temperature, rate of flow and aeration of the water being the same at the end as at the beginning of the period. Most of the fish showing rhythmic changes in activity followed patterns of this type, although the active phase varied considerably, sometimes being interrupted by several hours of quiescence during the day, sometimes beginning later in the day, and occasionally continuing well into the night. The most constant period of quiescence occurred between midnight and 4 A.M., which is in agreement with Spencer's (1939) observations.

The third type of behavior, diurnal quiescence and nocturnal activity, was found less frequently than the second, although it was not uncommon. An example is

shown in Figure 3, which is based on records of the activity of a 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. The room was not entered between 4 P.M. July 3 and 8 A.M. July 5, and aside from the daily changes in light the environmental conditions apparently remained constant throughout the period.

The patterns of rhythmic fish did not seem to be fixed, however, even when the environmental conditions remained unchanged. After several weeks of rhythmic behavior the fish frequently became arrhythmic for several weeks or months, occasionally becoming rhythmic again in the course of extended periods of observation. This suggests that those fish which did not show daily activity rhythms under the

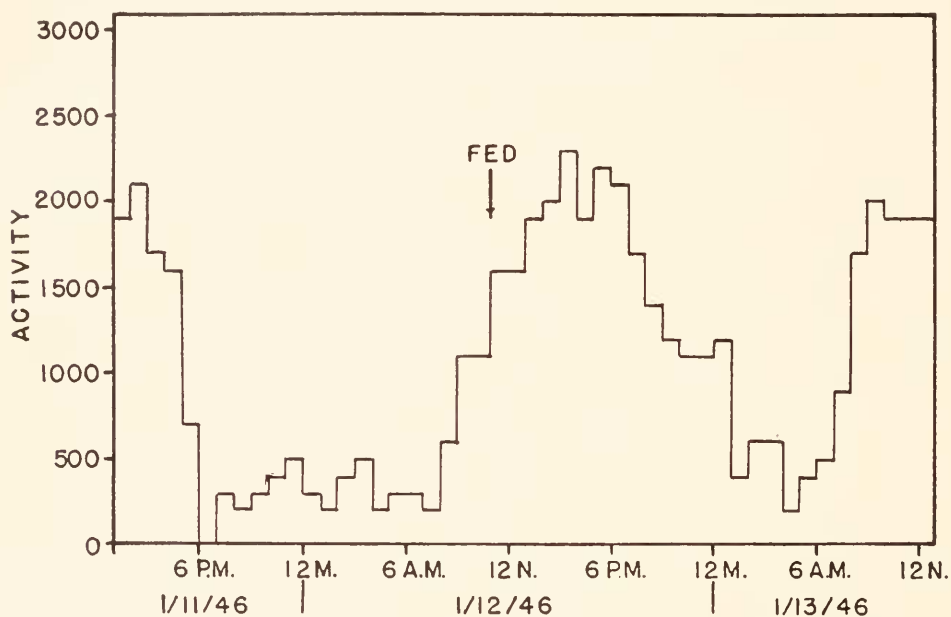


FIGURE 2. Activity pattern of 35-gram male goldfish between 1 P.M. January 11 and 1 P.M. January 13, 1946. Activity is expressed as number of impulses recorded each hour. Temperature 21.5° C. Fed at 11:05 A.M. January 12.

experimental conditions may have done so eventually had they been studied for longer periods, and that by chance the observations were made during arrhythmic periods.

Activity and food

The fish were fed rolled oats, commercial fish foods, shredded shrimp, ground liver or chopped earthworms about three times a week, usually 0.5 to 1 gram at each feeding. The effects of daily feeding, larger amounts of food and starvation were also studied. Under the conditions of the experiments the type of food given had no consistent effects upon activity, but the quantity of food had pronounced effects, particularly on the total amount of activity. A well fed fish was usually sufficiently active that the number of impulses recorded in the course of a 24-hour period averaged between 500 and 1,500 an hour, and averages in excess of 2,500 impulses an

hour were not uncommon. Starvation caused this rate to decrease markedly, sometimes to fewer than 100 impulses an hour, although as a rule the lowest rates did not appear until the fish had been starved for a week or so. No fish was observed to become completely inactive for periods of more than an hour or two, however, even when starved for two weeks. The effects of feeding after a period of starvation were striking, activity increasing to normal "well fed" rates within a few minutes. Doubtless the swimming movements associated with feeding accounted for some of the activity recorded following the introduction of food, but it seems that the nutritional state also affected the amount of activity. Food given in amounts of one

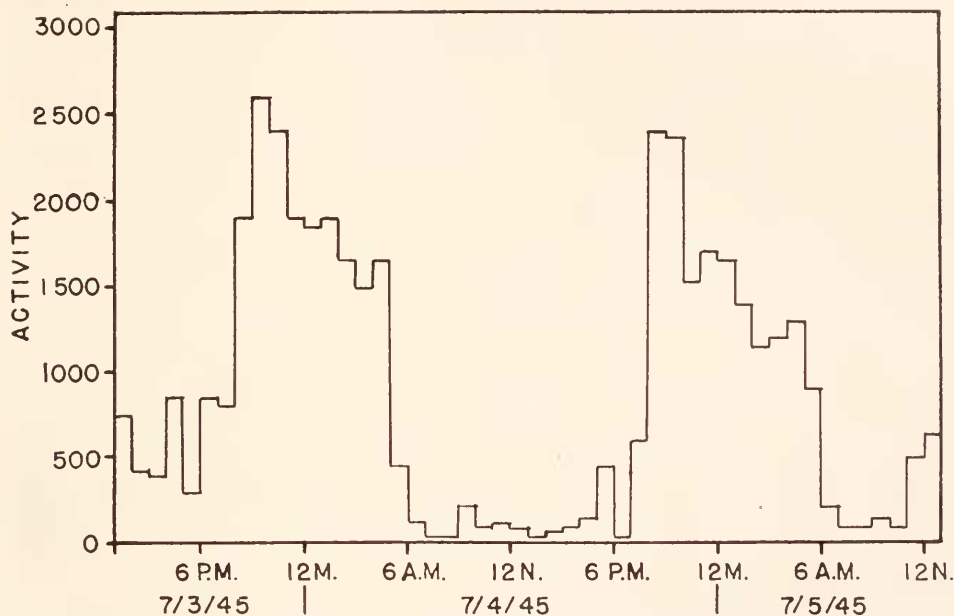


FIGURE 3. Activity pattern of 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. Units as in Figure 2. Temperature 23.5° C.

gram or less was usually consumed within three to six hours, but the fish remained active (in accordance with their activity patterns) for from several days to a week after they had been fed. Similarly, Spencer (1939) found the goldfish to maintain a high rate of activity for several hours after feeding, although in his experiments the food was usually consumed within 15 minutes or less.

The effects of feeding upon activity rhythms were not studied in detail, but the available data bearing on this question indicate that although the rhythms appearing under natural conditions of light were frequently modified by the quantity of food and the time of feeding, they were not causally related to food. Feeding modified the activity patterns of some fish for part or all of the subsequent 24-hour period, usually by prolonging the active phase. A response of this type may be seen in Figure 2. The fish was fed 0.5 gm. of rolled oats at 11:05 A.M. on January 12 (the previous feeding being on January 9); it will be noted that the activity level remained relatively high for a much longer period on the night of January 12 than on

the preceding night. On the other hand some fish showed no change in activity in response to feeding, provided of course that they had not been starved. Variations in the quantity of food and in the time and frequency of feeding did not seem to have permanent effects on the activity rhythms, and feeding at the same time each day did not cause arrhythmic fish to become rhythmic.

Activity and light

The goldfish did not seem to be much affected by changes in light intensity while they were not following daily activity rhythms, but they were usually quite responsive to light during their periods of rhythmic behavior. In fact, when the fish were well fed and undisturbed the activity rhythms seemed to be closely related to the daily changes in natural light, as Szymanski (1914) has reported previously. This view is supported by several observations in addition to the fact that the active and quiescent phases of the cycles usually coincided with day and night. Periods of nocturnal activity and diurnal quiescence were shown by the 32-gram male goldfish mentioned above in July and December of 1945. Although the water temperature and other factors except light were the same during both periods, the nocturnal phase of activity usually began earlier in the evening (between 5 and 6 P.M.) and ended later in the morning (between 7 and 8 A.M.) in December than in July, when it usually began between 7:30 and 8:30 P.M. and ended between 5 and 6 A.M. This suggests of course that the nocturnal phase of activity was limited by the setting and rising of the sun. This fish also responded readily to experimental changes in light intensity, particularly during the day, when darkening the chamber caused its activity to increase to levels usually reached only at night. Records were also obtained in which diurnally active and nocturnally quiescent fish remained active on nights when bright moonlight entered the room in which they were kept. Spencer (1939) found that the regular diurnal rhythm of the goldfish could be obliterated by covering the tank by day and lighting artificially at night. This procedure was accompanied by night feeding, however, so that the change in activity may not be attributed solely to the reversed lighting.

On the basis of these observations attempts were made to maintain goldfish at definite rates of activity by exposing them to continuous dim light and to continuous darkness for periods lasting as long as three weeks, but without success. The fish did not maintain constant rates of activity under either condition, but continued to alternate periods of increased activity with periods of relative quiescence. In order to maintain a low rate of activity it was necessary to starve the fish for about a week, the relationship between nutritional state and amount of activity being similar to that described in the preceding section.

Activity and disturbance

The goldfish proved to be extremely sensitive to disturbances. Noise, slight changes in the water level, sudden lights, the mere presence of the observer in the room, or such minor disturbances as the quiet opening and closing of the door to the room usually caused a change in the rate of activity. Fish that had been active before the disturbance almost invariably became less active, sometimes practically motionless, while quiescent fish frequently, although less consistently, became active when disturbed. Whichever the response, the original state of activity was

usually resumed within a few minutes after the disturbance had ceased. The degree of response seemed to be related to the amount of disturbance, for when the observer moved slowly and quietly the change in activity was usually less pronounced, and recovery more rapid, than after ordinary passage through the room or adjustment of the apparatus. The effects of disturbances upon the activity of an otherwise quiescent fish may be seen in Figure 3. The room was entered several times in the course of the afternoon of July 3 and on July 5, although the experimental chamber was not approached and the fish could not see the cause of the disturbance. It is obvious that the rates of activity were higher than at corresponding hours on July 4, when the room was not entered. Such sensitivity has been observed in other species of fish by Clausen (1934), who found that a shadow passing over the aquarium caused increases in the body temperatures of perch and members of the sunfish group.

THE RELATIONSHIP BETWEEN ACTIVITY AND OXYGEN CONSUMPTION

Method

The activity and corresponding oxygen consumption of individual goldfish were measured in observation periods ranging in length from 11 to 210 minutes. Activity was measured in terms of the number of impulses recorded in a given period, and the amount of oxygen consumed by the fish in that period was determined by means of a continuous flow system. A control chamber similar to the experimental and housed in the same case was supplied with a continuous stream of water from the reservoir supplying the fish. The water in each chamber was covered with a layer of heavy paraffin oil 2.5 cm. thick to retard the diffusion of oxygen from the air, and a sample of the effluent from each chamber was analyzed for oxygen by the Winkler method at the beginning and end of each period. The samples were collected in narrow necked glass stoppered bottles of about 270 cc. capacity arranged to serve as constant level drains (Fig. 1). Each line was arranged so that the water passed through the outlet siphon to the bottom of the sampling bottle and overflowed into a funnel so that it could be collected for flow rate determinations. Although the rates of flow ranged from 70 to 100 cc. a minute in the course of the study, the rate for any one day's series of samples was held practically constant. Due care was taken to prevent the diffusion of oxygen into the samples and to obtain representative samples from experimental and control lines. Samples that were contaminated by particulate matter were discarded. The permanganate modification was used in most of the analyses, but was omitted during some of the shorter periods. The results obtained with and without the modification were quite similar, however, which was not unexpected in view of the fact that from four to six liters of water passed over the fish each hour.

The volume of water flowing through the system in the course of an observation period being known, together with the oxygen content of the water leaving the control and experimental chambers at the beginning and end of that period, the oxygen consumed by the fish could be calculated. The calculations took into account the change in the amount of oxygen in the constant volume of water in the chamber. The volume of water displaced by the fish was too small to affect the calculations.

The samples were collected with the foregoing observations on activity patterns

and modifying factors in mind, the periods being timed to yield data at the activity rates desired, and the method of sampling being modified as necessary to minimize disturbance of the fish. In the latter connection the outlet tubes were lengthened so that samples were collected about 10 feet away from the chambers, and the room was not entered except for sampling and rate of flow determinations. Precautions were taken to prevent changes in the water level in the experimental chamber as there were indications that small changes in the level stimulated the fish. These precautions were necessary also because the volume of water in the chamber, as well as that flowing through it, entered into the calculations of oxygen consumption. Samples were discarded if subsequent examinations of the activity records showed that they had been collected while the fish was undergoing marked changes in activity as a result of disturbance or in accordance with an activity rhythm. This was necessary because although the activity record was instantaneous the change in the oxygen concentration of the samples tended to lag somewhat behind that in the chamber, the sample drawn at any instant representing the average of the water flowing into the bottle in the few minutes preceding its removal. The temperature of the water was recorded for each observation period in order to avoid discrepancies attributable to the effect of temperature on metabolic rate (Ege and Krogh, 1914).

Owing to its viscosity and the accumulation of emulsified oil and water at the oil-water interface, the layer of oil interfered with the movements of the paddle shaft. Its thickness was therefore reduced to 1 cm. within the central glass tube, thus permitting the paddle to move about as freely as with a water surface. This tube extended below the interface far enough to prevent the emulsion from accumulating around the paddle shaft. The oil within the tube had to be changed now and then, however, to remove the small amount of debris that entered it from beneath. The detector contacts and bakelite rod were cleaned every few days as a precaution against their becoming coated with oil, which seemed to spread slowly up the paddle shaft.

It was established by appropriate tests that the layer of paraffin oil was effective in preventing the diffusion of significant amounts of oxygen into the water from the atmosphere. In no test did the apparent leakage exceed the limits of error of the Winkler method itself (Allee and Oesting, 1934), and it was usually considerably less. The average apparent rate of change for the contents of the experimental chamber was 0.0015 cc. of oxygen a minute, which was so much smaller than the rate at which the fish consumed oxygen that even had the apparent change been real it would have had but little effect on the results. It should be mentioned in this connection that the oil layer was disturbed relatively little by the movements of the fish, inasmuch as the wire screen kept the fish out of the oil and glass plates resting on this screen lessened the churning effects of the water beneath it.

Results

Three goldfish were studied at several temperatures in a total of 104 observation periods. As the results on all three were much alike, data on but one of the fish, a 32-gram male on which over two-thirds of the measurements were made, are presented here.

The relationship between activity and oxygen consumption at temperatures between 23 and 25° C. is shown in Figure 4, in which oxygen consumption in cubic

centimeters per minute is plotted against activity in impulses per minute. Fifty-nine observation periods are represented, each point corresponding to one period. The line merely indicates the trend, and has not been fitted to the data mathematically. As was to be expected, oxygen consumption and activity proved to be closely related, the relationship apparently being linear above the basal level of oxygen consumption. Although the values for oxygen consumption at any one rate of activity are seen to vary somewhat, the trend is clear cut: at high rates of activity the rate of oxygen

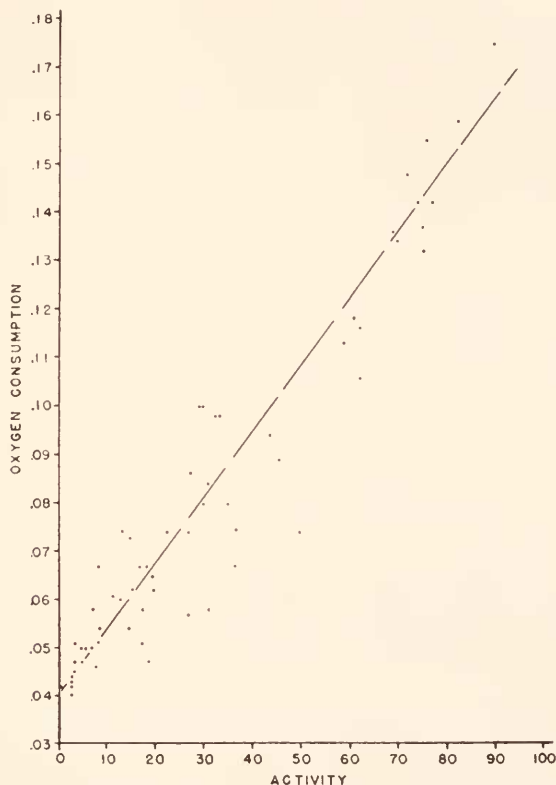


FIGURE 4. Activity and oxygen consumption of 32-gram male goldfish. Activity in impulses/minute; oxygen consumption in cubic centimeters/minute. Temperature 23 to 25° C.

consumption is correspondingly high; at low activity rates less oxygen is consumed. The discrepancies that do occur may well have been due to errors in measurement, rather than to a lack of correspondence between activity and oxygen consumption. In this connection the data on oxygen consumption follow those on activity quite closely when the comparison is restricted to one day's series of measurements, thus ruling out discrepancies attributable to slight differences in the adjustment of the detector contacts. Such a series is shown in Figure 5, which is based on data obtained with the same fish in a series of thirteen consecutive 15- to 25-minute periods at 22° C.

According to the slope of the data shown in Figure 4, the basal oxygen con-

sumption of this fish was in the vicinity of 0.040 cc. a minute, or 0.075 cc. per gram per hour.

DISCUSSION

The results of the present study have a bearing on the collection and interpretation of data on the respiratory metabolism of fishes, and in the light of these results the method described seems to offer a number of advantages not found in previous methods which have been employed for this purpose.

The advantages of the continuous flow method for measuring respiratory metabolism in fishes have been discussed by Keys (1930) and need not be reviewed here. In view of the relationship between oxygen consumption and activity, however, the

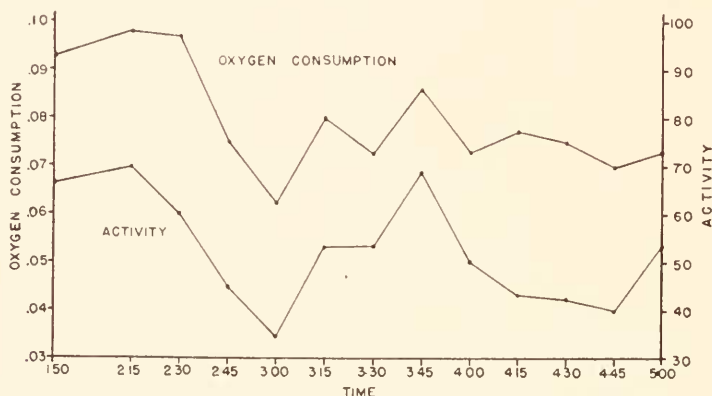


FIGURE 5. Activity and oxygen consumption of 32-gram male goldfish in each of thirteen consecutive observation periods between 1:35 P.M. and 5 P.M. November 13, 1945. Units as in Figure 4. Each point on the upper line represents the average rate of oxygen consumption for the 15- or 25-minute period preceding it. Each point on the lower line represents the average rate of activity for the corresponding period.

observations on the effects of disturbances may be applied to the use of this method, inasmuch as the process of sampling may disturb the fish. Should a change in the rate of activity (and consequently of oxygen consumption) occur at the time of sampling, the sample would not be representative of the volume of water and unit of time to which it is related in the calculations. The resulting error could be of considerable importance, particularly in investigations in which the samples consisted of water flowing directly from the experimental chamber and overflowing through a sampling bottle. This source of error has been recognized of course, and in some investigations the experimental chamber has been covered in attempts to minimize stimulation of the fish. It seems very doubtful, however, whether covering a goldfish so that it cannot see the investigator is an adequate safeguard against disturbance. One advantage of using an activity detector in the continuous flow method then lies in the fact that any sudden change in activity occurring at the time of sampling can be detected, so that the reliability of the sample may be judged. Furthermore, the activity record can be used to test the effectiveness of the steps taken to avoid disturbance.

It is of course well known that fluctuations in activity during the test periods constitute a major obstacle to the correct interpretation of measurements of oxygen consumption, and numerous attempts to overcome this difficulty have been described (Ege and Krogh, 1914; Hall, 1929; Adkins, 1930; Keys, 1930; Wells, 1932, 1935; Clausen, 1933; Smith and Matthews, 1942). These measures include the use of narcotics, observing that the fish remains quiet, maintaining constant conditions of light, sampling at the same time each day, restricting the movements of the fish, and maintaining the fish in an experimental chamber until it appears to have come to rest or at any rate to have reached a steady state. Although such measures may permit the establishment of the reality of a change in oxygen consumption in connection with an experimental procedure, they do not appear to give a completely satisfactory basis for the interpretation of that change. The interpretation must be based on knowledge of the activity of the fish, inasmuch as oxygen consumption is affected by changes in the basal metabolic rate as well as by activity. The method employed must therefore be capable of supplying information on activity and oxygen consumption at the same time, so that the fraction of the respiratory exchange associated with basal metabolism may be distinguished from that due to muscular activity (Wells, 1935). None of the above methods seems to be adequate for this purpose.

Narcotics are of doubtful value in studies of this type, even for measuring basal metabolic rate alone (Adkins, 1930). Among other objections are indications that an important fraction of the metabolic functions of the fish may be suppressed to such an extent that the oxygen consumption falls below the basal level as it is generally understood (Keys and Wells, 1930). In fact, Ege and Krogh (1914) considered it necessary to use artificial respiration to insure the survival of their goldfish, the narcotic having interfered with normal respiratory movements. The other methods are open to criticism because they are based on the assumption, rather than the knowledge, that the fish is quiescent or at a constant level of activity under the conditions of the experiment. The results of the present work suggest that for the goldfish at any rate this assumption may be unwarranted. The fact that a goldfish is quiescent while it can be seen should not be taken as proof that it remains so while unobserved, and it does not seem justifiable to assume that constant environmental conditions mean constant rates of activity. So far as the goldfish is concerned, the individual variations in activity open to question the reliability of methods based on sampling at the same time each day, particularly if several fish are being compared. Confining the fish to a small respiration chamber to restrict its movements gives no assurance that it will remain quiescent or even at a constant rate of activity, and the fact that the oxygen consumption varies over a wide range in such chambers supports this objection. This method would seem to have a further disadvantage for measuring the basal metabolic rate in that a fish confined to a small tube must swim continuously, however slowly, in order to maintain its position in the current. The practice of leaving the fish in the respiratory chamber until its oxygen consumption has reached a relatively low and constant rate (Keys, 1930; Wells, 1935) is far superior to the earlier techniques, but it is limited in its application by the fact that it gives no information as to the amount of activity associated with the steady state.

The requirements of a satisfactory method appear to be met by combining an activity detector with the continuous flow system. The rate of activity can then be

measured at the same time that oxygen consumption is determined, and the results interpreted accordingly. As the activity record is continuous, periods of quiescence can be selected for measuring basal oxygen consumption, so that it is not necessary to employ special techniques designed to control activity. In this connection, however, starvation may be used as a means of prolonging the quiescent state. A further advantage of the present method lies in the fact that the fish can be maintained in good health in the experimental chamber for months, so that measurements of its respiratory metabolism need not be obscured by the excitement and other effects of handling.

SUMMARY

1. Apparatus for making continuous records of the activity of isolated and undisturbed goldfish is described, together with a method for measuring oxygen consumption and activity simultaneously.

2. The goldfish were quite variable in their patterns and rates of activity under the experimental conditions. Some fish were diurnally active and nocturnally quiescent, others followed the opposite pattern and still others were arrhythmic throughout the periods during which they were observed. Moreover, some fish showed both rhythmic and arrhythmic states of activity when studied for periods extending over several weeks or months.

3. Food, light and minor disturbances had pronounced effects on the activity of the goldfish.

4. Simultaneous measurements of oxygen consumption and activity are presented which indicate that the two are closely related above the basal level of oxygen consumption.

5. The bearing of these observations on the collection and interpretation of data on the oxygen consumption of the goldfish and on the measurement of its basal metabolic rate is discussed, and certain advantages of the method are described.

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